The Division of the Collar-Cells of the Calcarea Heterocœla.

By
Muriel Robertson, M.A.

With Plate 19.

Introduction.

In 1910 Prof. Minchin and I described (5) the division of the collar-cells in the sponge Clathrina coriacea (Montagu) with a view to obtaining an insight into the behaviour of the basal granule of the flagellum during cell-division. In Clathrina coriacea and the Clathrinidæ generally the nucleus lies at the base of the collar-cells, and, just before division, comes to the apex, which is the position it occupies in the embryo. In the Leucosolenidæ and most Heterocœla, on the other hand, the nucleus lies towards the apex thoughout the whole life of the sponge, and the flagellum arises from a granule which is only very little removed from the nucleus, and attached to it by a double rhizoplast. At Prof. Minchin's suggestion I have recently investigated the division of the collar-cells of two members of the Heterocœla, namely, Grantia compressa and Sycon sp.,[1] to see how the process compares in the two families.

It was at first expected that the basal granule or blepharophast of the flagellum in Grantia and Sycon would lie actually in the nucleus, but that is not the case; the blepharoplast in the vegetative condition of the cell is placed at the apical margin, and is connected with the nucleus by a

[1] Either Sycon ciliatum or S. coronatum; it was not possible to determine the species, since the methods of preservation used were such as dissolved the spicules.

double rhizoplast (figs. 1–5, 21). One is therefore here, as in Clathrina, dealing with an extra-nuclear structure. Both in the earlier paper published with Prof. Minchin and in the present account the work has been done with the view of elucidating the blepharoplast-centrosome question, and not from the standpoint of the morphology of the sponge.

The literature of the subject was fully discussed in the earlier paper, and I propose only to consider here a few additional publications which have particular bearing on the questions under investigation. These will be treated under the "General considerations" at the end of the paper.

Material.

The material was obtained from the Marine Laboratory at Plymouth, and was fixed in various ways. Corrosive sublimate and acetic acid, Flemming's fluid and Bouin's fluid were all used; of these Bouin's fluid gave unquestionably the best result. The stains used were Heidenhain's iron-hæmatoxylin; iron hæmatein, according to Dr. Seidlin's method ('Parasitology,' vol. iv, p. 94), and Twort's combination of neutral red and Lichtgrün; these all gave good results, and were used with good effect to supplement and control each other.

The collar-cells of these sponges form, as is well known, a single layer of epithelium lining the radial tubes or flagellated chambers. The shape of the cell is subject to considerable variation, according to the exact condition of the sponge, and, indeed of the different parts of the sponge, at the moment of fixation; they are, however, usually either flask-shaped (fig. 1), or shaped like chimneys (fig. 2). The collar is a delicate tubular structure which projects from the free end of the cells, and varies somewhat in length according as the cell is fully extended or not. These structures are of extreme delicacy, and are easily destroyed by unsuitable fixation.

The cytoplasm of the cell is granular and vacuolated, and may show large inclusions. The nucleus lies a little below the

apex, and though usually spherical, may sometimes be slightly drawn out towards the top of the cell. The blepharoplast is a small granule staining intensely with iron-hæmatoxylin; with iron-hæmatein it shows quite sharply, but is pale grey in colour, while the chromatin of the nucleus is a blueish-black; with Twort's stain it is not very readily visible, but whenever it can be made out it is always of the cytoplasmic colour, namely, green (fig. 28). There is no shred of evidence in the staining reaction to suggest that the structure in question is of a chromatic or nuclear nature. To insist on the achromatic nature of a centrosome (as in effect the blepharoplast of a collar-cell really is) at the present date seems a useless waste of energy and almost an anachronism, were it not for the recent theories of such well-known workers as Hartmann and Prowazek (2a). The centrosome-blepharoplast is situated at the extreme upper edge of the protoplasmic body, and is connected, as has already been said, with the nucleus by a double rhizoplast. In many cases the double nature of this last structure escapes observation, the two strands lying very closely side by side. It is, however, obvious from a careful study of a sufficiently large number of cells that two strands (sometimes widely separated from each other and forming a triangle as in figs. 3 and 5) and not one only connect the blepharoplast with the nucleus. This rhizoplast persists throughout the whole life of the cell except, as will be seen hereafter, for a very short period immediately before division. From the blepharoplast arises the flagellum, which is of considerable length and extends beyond the collar.

To talk of the nucleus of any living and functioning cell as being in the resting state is a self-contradictory phrase which cytologists are only gradually abandoning. In the collar-cells the variety of appearance in the nucleus of the non-dividing cells is particularly striking. Unfortunately from the nature of the case one is unable to correlate the physiological state and the particular appearance of the nucleus, and of the distribution of the chromatin, etc., within

it. One or two points can, however, be observed. It has been noticed that the nucleus immediately before division always shows a dense staining karyosome which disappears during the formation of the primitive spireme (fig. 9). There is, of course, no proof of the converse, and it by no means follows that because the nucleus shows a dense karyosome it is therefore about to enter upon division. In the work on Clathrina a corresponding point was observed, namely, that cells about to divide showed a pale nucleus containing a dense karyosome. Another point in the life of the cell can, as it were, also be caught, and that is the period immediately after division. The young daughter-cells have certain characteristic features to be noted later, and can readily be recognised. The nucleus in these cells is always of the reticulate type, and does not show the karyosome (fig. 21). Whether this behaviour on the part of the karyosome may prove of importance when we know more of what induces a cell to divide cannot be determined at present, but is worth recording in passing.

Mitosis.

The first alteration to be observed in a collar-cell about to divide is usually the disappearance of the flagellum and the subsequent division of the blepharoplast, which has at this stage lost its connection with the nucleus. The exact sequence of these early processes is, however, subject to a good deal of variation, and sometimes the flagellum is retained until after the division of the blepharoplast (fig. 10). In the nucleus itself very characteristic changes take place, culminating in a curious phase which is of very constant occurrence and which corresponds to the spireme-stage. The dense karyosome gradually breaks up (figs. 3–10), and there are formed a number of masses of chromatin which become increasingly definite in appearance; they are connected together at this stage by delicate filamentous strands which do not take up the chromatin stains. This condition corresponds to the spireme-stage, which never reaches a

greater development than this in the case of collar-cells; a glance at figs. 6 and 10 will make it clear how far removed it is in appearance from the long continuous coiled thread so universally seen in more developed types of karyokinesis. Speaking generally, the blepharoplast divides just before the above changes take place in the nucleus, but here again slight variations in the time-relations are very often to be observed. The blepharoplasts gradually move apart, but may sometimes be joined for a time by a strand; this stage is of rare occurrence and is illustrated in fig. 8. More often the blepharoplast divides and the daughter-blepharoplasts move apart without the junction between them persisting. These two little granules are destined to play the part of centrosomes in the coming mitosis, but do not show the radiations passing out from them which are so characteristic of most centrosomes. As time goes on they come to lie on either side of the nucleus, and a spindle-apparatus is formed between them. In fig. 11 an interesting stage is shown where the spireme is not yet quite complete and the first signs of the spindle can be seen arising between the centrosomes on either side of the nucleus. The mitosis proceeds and the equatorial plate is formed, but the chromosomes are not very distinct. At this stage there grows out from the centrosomes on either side the first rudiment of the new flagella (figs. 12–16 and 21), thus exactly repeating the state of affairs observed in Clathrina coriacea. The diaster-stage (figs. 17–19) follows in due course, and the two poles of the spindle each with its mass of chromatin curve upwards through approximately a right angle (figs. 20 and 21).

The centrosome-blepharoplast is still connected with the chromatin mass by a double strand (figs. 20, 21), which is the remains of the spindle. This connection persists as the double rhizoplast already noted and only disappears again just before the next division. In Clathrina this junction disappears and the blepharoplast is completely cut adrift from the nucleus during the vegetative life of the cell. The

two dense chromatin-masses of the diaster-stage re-form into the two nuclei of the daughter-cells. The spireme is entirely suppressed at this point, and the reconstituted nuclei are, as has already been noted, of the reticulate type (figs. 22, 23). The young cells are narrower and project forward beyond the epithelium, and the nuclei appear for a time to be rather smaller than those of the neighbouring cells. The collar disintegrates at an early stage and is re-formed anew in each of the daughter-cells. It is interesting to note in passing that, although this is a quite typical metazoan mitosis, it reveals one or two rather primitive features, such as the very slight development of the spireme, the absence of rays from the centrosomes, and the general indistinctness of the chromosome.

General Considerations.

The main feature of interest in the foregoing account is that we have here another instance of a blepharoplast playing the part of centrosome, and the chief importance of the observation lies in the evidence it brings as to the simple achromatic nature of this structure. In a recent paper Hartmann and Chagas (**1**) describe the division of a number of free-living flagellates, and some of the forms they deal with are of particular interest.

The part of their work that bears most immediately on the question under consideration is that which treats of the division of Spongomonas uvella and Spongomonas splendida. Here two flagella are present in each individual, and there are two blepharoplasts which are generally not connected in any way with the nucleus and resemble in this particular the condition found in the collar-cells of Clathrina coriacea. The authors are inclined to think that this is due to a secondary absorption of the rhizoplast, and is, as it were, a late condition in development. They are led to this conclusion by the fact that immediately after division the cells of Spongomonas show a quite clear connection between the blepharoplast and the karyosome of the nucleus.

In these forms there is a centriole present in the karyosome. At division the centriole divides into two parts joined by a centrodesmose, and a fairly well-developed mitotic spindle is formed with the centrioles at the two poles and the chromatin arranged in an equatorial plate at the centre. The centrioles generally divide a second time during the course of mitosis, the two granules thus formed lying close beside each other in each case. The behaviour of the flagella is of particular interest; the old flagella are thrown off very early at the outset of division. In the majority of cases the new flagella arise after the completion of mitosis by what the authors call the heteropolar division of the reconstructed karyosomes. It is best to quote the description of the subsequent events in the authors own words: "Da schon bei der Mitose Dopplecentriolen vorhanden waren, sind zwei Möglichkeiten denkbar; entweder sind dieselben bei dieser heteropolaren Teilung einfach verteilt worden, wobei der eine Pol (Basalkörper) zur Bildung des Diplosoms sekundär eine zweite Teilung erfahren musste; oder aber die doppelten Centriole teilen sich gleichzeitig und bilden so direkt das Diplosom. Das zurückgebliebene Doppelcentriol würde dann im Ruhestadium des Kernes infolge fester Aneinanderlagerung scheinbar als einfaches Centriol erscheinen. Bei beiden Möglichkeiten stimmt der Modus der Geisselentstehung prinzipiell mit dem von Cercomonas überein. Daneben kommt aber noch eine sehr interessanter zweiter Modus vor, indem schon im Stadium der Æquatorialplatte die neuen Tochtergeisseln von den Centriolen aus gebildet werden" (pp. 81, 82).

The above description is of great interest and importance, and shows very clearly the centrosomic origin of the blepharoplast in the Protozoa under discussion. I should like, however, to point out that it is an unfortunate confusion of language for the authors repeatedly to talk of heteropolar mitosis of the karyosome when what they both figure and describe is the division of the centriole contained in the karyosome. The word "karyosome" as used in protozoolo-

gical literature means a structure composed of chromatin embedded in an achromatic substance, and which usually contains a centriole or centrosome. In general cytological writing the term is applied to a condensation of chromatin as distinguished from a true nucleolus. In neither sense are the authors justified in saying that the process they describe is really a heteropolar mitosis of the karyosome. The term "mitosis" implies some kind of a partition of all the substance of the structure involved, and heteropolar mitosis of the karyosome means that the plastin, chromatin, etc., have undergone an unequal division.

What these authors show in their excellent work on the two species of Spongiomonas and on Cercomonas parva described in the same paper is the splitting-off of a minute centrosome-like granule which is bound permanently (as in Cercomonas), or for a time (as in Spongiomonas), to the karyosome by a slender thread. The centriolar nature of these granules is abundantly demonstrated in the last sentence of the paragraph quoted above. It is clear that this process is essentially the same as that described in the collar-cells, and brings the blepharoplast of these Protozoa into line with those of the sponge-cells.

The main points raised in all this work are very clearly and broadly put in a valuable article (3) by Hertwig, who comes to the conclusion that the cytoplasm is a compound substance composed (A) of a substance very closely akin and practically identical with the achromatic contents of the nucleus, and (B) of a substance akin to chromatin, and from which this latter is built up. According to Hertwig's idea, the chromatic and achromatic substances are distinct and separated out from each other in the nucleus, while in the cytoplasm the same two substances are present in some sort of combination. The centrosome is for Hertwig simply "ein individualisiertes Stückchen achromatischer Kernsubstanz," and adds that he is prepared to admit that centrosomes may arise outside the nucleus from the achromatic substance of the cytoplasm. These conceptions of Hertwig's are of course to be considered as a

broad fundamental theory rather than as a complete explanation of all the facts. They have, however, the great merit of being formulated from physiological as well as morphological observations, and therefore pay due regard to the processes of cell-life. Very probably the cytoplasmic substance must be regarded as much more complex than is suggested in Hertwig's survey. It is interesting to note in this connection that the work of Reichenow (4) has shown that the substance called volutin, which arises in the cytoplasm, is a stage in the building up of the nuclear chromation—or to put the matter more precisely, the chromatin in the nucleus increases at the expense of the volutin in the cytoplasm. The point in this description of Hertwig's view that I wish to emphasise is that it brings out very clearly the achromatic nature of the centrosome. In a later part of the same article he goes on to say that "die Centrosomen und die Basalkörperchen von Wimpern, Geisseln und Pseudopodien analoge Gebilde sind." The proposition embodied in the last sentence has received additional proof and has been further extended by practically all the recent work upon the subject.

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Literature.

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2a. Hartmann and Prowazek.—"Blepharoplast, Caryosom, und Centrosom," 'Arch. f. Protistenkunde,' Bd. x, 1907.

3. Hertwig.—"Die Protozoen und die Zelltheorie," 'Arch. f. Protistenkunde, Bd. i, 1910.

4. Reichenow.—"Hæmogregarina stepanowi," 'Arch. f. Protistenkunde, Bd. xx, Heft 3, 1901.

5. Robertson and Minchin.—"The Division of the Collar-cells of Clathrina coriacea," 'Quart. Journ. Micr. Sci.,' vol. 55, part 4, November, 1910.

EXPLANATION OF PLATE 19,

Illustrating Miss Muriel Robertson's paper on "The Division of the Collar-cells of the Calcarea Heterocœla."

[The figures are drawn with the aid of the camera lucida at a uniform magnification of 2000 linear.[1]]

Fig. 1.—Collar-cells of Grantia showing the flask shape. Note the karyosome in the nucleus.

Fig. 2.—Collar-cells of Grantia showing the elongated chimney shape.

Fig. 3.—Collar-cell with reticulate nucleus. It also shows the double rhizoplast forming the connection between the blepharoplast and the nucleus (Grantia).

Fig. 4.—Collar-cell of Sycon showing pale nucleus with deeply staining karyosome.

Fig. 5.—Collar-cell of Grantia showing reticulate nucleus with karyosome. Note the double rhizoplast.

Fig. 6.—Early spireme-stage (Grantia).

Fig. 7.—Stage very like that shown in fig. 6, but the spireme is in a still earlier condition (Grantia).

Fig. 8.—Precocious division of the blepharoplast; the specimen is fixed with Flemming's solution (Grantia).

Fig. 9.—Early stage in division; the blepharoplast is newly divided, and the spireme is just being formed at the expense of the karyosome. The flagellum has been thrown off (Sycon).

Fig. 10.—Slightly later stage; the blepharoplast has divided but the flagellum is still retained (Sycon).

Fig. 11.—Slightly aberrant stage; the blepharoplast - centrosomes occupy either end of the spindle which is just forming, while the spireme-stage has not yet been completed (Grantia).

Fig. 12.—Equatorial plate-stage with the chromosomes just splitting. The first rudiment of the new flagellum is visible at one pole (Grantia).

Figs. 13 and 14.—Equatorial plate-stages (Sycon).

Fig. 15.—Equatorial plate showing flagellum growing out from either centrosome (Sycon).

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[1] I am indebted to Miss Mabel Rhodes for the care and skill with which she has executed the figures.

Fig. 16.—Equatorial plate showing remnant of collar (Grantia; Flemming fixation).

Fig. 17.—Very early diaster-stage (Grantia).

Figs. 18 and 19.—Diaster-stages (Grantia and Sycon).

Figs. 20 and 21.—Division of cells. Note the junction between the nuclei in course of reconstruction and the blepharoplasts (Grantia).

Figs. 22 and 23.—Newly divided cells. Note the condition of the nucleus. Both the cells and the nuclei are below the normal size (Sycon).

Figs. 24–27.—Stage of division stained with Twort's stain. Note the faint green colour of the blepharoplast-centrosomes (Grantia).

Fig. 28.—Vegetative stage stained with Twort's stain (Grantia).